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Responses of plant diversity and soil microorganism diversity to nitrogen addition in the desert steppe, China

YE He[1,2], HONG Mei[1,2*], XU Xuehui[1,2], LIANG Zhiwei[1,2], JIANG Na[1,2], TU Nare[1,2], WU Zhendan[1,2]

[1] College of Grassland, Resources and Environment, Inner Mongolia Agricultural University, Inner Mongolia Key Laboratory of Soil Quality and Nutrient Resources, Hohhot 010018, China;
[2] Key Laboratory of Agricultural Ecological Security and Green Development at University of Inner Mongolia, Hohhot 010018, China

Abstract: Nitrogen (N) deposition is a significant aspect of global change and poses a threat to terrestrial biodiversity. The impact of plant-soil microbe relationships to N deposition has recently attracted considerable attention. Soil microorganisms have been proven to provide nutrients for specific plant growth, especially in nutrient-poor desert steppe ecosystems. However, the effects of N deposition on plant-soil microbial community interactions in such ecosystems remain poorly understood. To investigate these effects, we conducted a 6-year N-addition field experiment in a *Stipa breviflora* Griseb. desert steppe in Inner Mongolia Autonomous Region, China. Four N treatment levels (N0, N30, N50, and N100, corresponding to 0, 30, 50, and 100 kg N/(hm^2•a), respectively) were applied to simulate atmospheric N deposition. The results showed that N deposition did not significantly affect the aboveground biomass of desert steppe plants. N deposition did not significantly reduce the alfa-diversity of plant and microbial communities in the desert steppe, and low and mediate N additions (N30 and N50) had a promoting effect on them. The variation pattern of plant Shannon index was consistent with that of the soil bacterial Chao1 index. N deposition significantly affected the beta-diversity of plants and soil bacteria, but did not significantly affect fungal communities. In conclusion, N deposition led to co-evolution between desert steppe plants and soil bacterial communities, while fungal communities exhibited strong stability and did not undergo significant changes. These findings help clarify atmospheric N deposition effects on the ecological health and function of the desert steppe.

Keywords: soil microorganisms; plant-microbial community interaction; plant diversity; nitrogen deposition; desert steppe

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1 Introduction

Desert steppes are unique transitional ecotones located between steppe and desert regions in Eurasia (Chen et al., 2017; Wu et al., 2021). The desert steppe of Inner Mongolia Autonomous Region, China has a simple plant community structure with limited water resources and poor soil nutrients. Therefore, it is considered a fragile ecosystem, especially under the background of global climate change (Angerer et al., 2008). In recent years, global climate change has led to an

*Corresponding author: HONG Mei (E-mail: nmczhm1970@126.com)
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increasing atmospheric nitrogen (N) deposition trend in China (Liu et al., 2013), and the average amount of N deposited in China was estimated at 20.4 (±2.6) kg N/(hm^2·a) during 2011–2015 (Yu et al., 2019). N deposition has been considered the third greatest threat to global terrestrial biodiversity (Payne et al., 2017). According to previous studies, atmospheric N deposition negatively affects the plant and soil microbial community diversity in terrestrial ecosystems (Bobbink et al., 2010; Zhang et al., 2018). Biodiversity is not only inherently precious but also bolsters the functioning abilities of ecosystems and provides reliable ecosystem services on Earth (Borer et al., 2014; Hautier et al., 2014; Yang et al., 2019). To maintain biodiversity, there is an urgent need to understand the responses of biodiversity to long-term N deposition in desert steppe ecosystems (Yang et al., 2019).

N deposition usually leads to an increase in plant productivity but a decrease in plant diversity. With some plant species becoming rare, N deposition can even result in species extinction (Stevens et al., 2004; Hautier et al., 2009; Yu et al., 2019). N deposition affects plants through many factors, such as nutrient imbalances and interspecific competition (Payne et al., 2017). Additionally, shifts in plant communities do not occur in isolation, and plant composition changes can influence the soil bacterial and fungal communities through cascade effects (Erisman et al., 2013; Payne et al., 2017). However, soil microbial communities can also influence plant fitness in various ways, such as the decomposition, nutrient cycling, and nutrient acquisition processes (Berg and Smalla, 2009; Schlatter et al., 2015). The interactions between plants and associated soil microbial communities are so intertwined that they can be considered as a whole entity that is subjected to environmental changes and selection (Zilber-Rosenberg and Rosenberg, 2008; Panke-Buisse et al., 2015; Hortal et al., 2017). A previous study showed that plant productivity is positively correlated with the proportion of soil microorganisms (Fan et al., 2020). Main microbial taxa are highly connected, which exert a considerable influence on microbiome structure and functioning, either individually or in a guild, irrespective of their spatial or temporal abundance (Banerjee et al., 2018). These microbial species often coexist in space and time and form clusters in microbial ecological networks; these clusters have been proven to provide nutrients for specific plant growth, especially in nutrient-poor desert steppe ecosystems (Delgado-Baquerizo et al., 2020).

N is an essential element for plant growth, and plants and microbes compete for N in soil. Increased plant-microbial competition for N can result in decreased soil N availability and microbial activity (Hu et al., 2001; Dunn et al., 2006; Shao et al., 2018). For instance, an increase in N can depress soil microbial activity by altering the metabolic capabilities of soil bacterial communities (Ramirez et al., 2012). Additionally, accumulated evidence suggests that N deposition can reduce the microbial biomass and change the microbial community compositions in diverse ecosystems (Treseder, 2008; Contosta et al., 2015). Increased N availability reduces the fungal biomass via changes in plant-specific exudates and alterations in nutrient competitions between plants and rhizosphere microbes (Bardgett et al., 1999; Zhang et al., 2018). Many ecological studies have highlighted the importance of plant-soil microorganism feedbacks and shifts in feedback effects associated with soil microbial community compositions, as these processes affect the coexistence and community compositions of plants (Bever, 2003; Reynolds et al., 2003; Lau and Lennon, 2011). Although the effects of plant-soil microorganism interactions on plant ecology have been widely studied, little research has addressed how belowground microbial communities influence plant biomass in nutrient-poor desert steppes (Kardol et al., 2007; Lau and Lennon, 2011; Hortal et al., 2017; van der Putten, 2017). Additionally, the ways in which microbial communities affect plant biomass are also unknown. In this study, we established a 6-year simulated N deposition experiment in the desert steppe region of Inner Mongolia Autonomous Region, northern China. Plant community productivity, soil physical-chemical properties, together with soil microbial groups and their roles were studied to evaluate plant-soil-microbiome interactions under N deposition.

2 Materials and methods

2.1 Experimental design

A field experiment was conducted in the Siziwang Banner (41°46′43″N, 111°53′41″E; 1456 m a.s.l.), an arid area in Inner Mongolia Autonomous Region, northern China. The average annual precipitation is 280 mm, with cumulative precipitation falling during the growing season (May–October) and constituting approximately 70% of the total precipitation throughout the whole year. The annual average temperature is 3.4°C. The soil in the study area has a sandy loam texture and is classified as a Haplic Calcisol by the Food and Agricultural Organization (FAO) soil classification system of the United Nations. The plant community is dominated by *Stipa breviflora* Griseb., *Neopallasia pectinata* (Pall.) Poljak., *Artemisia scoparia* Waldst. et Kit., *Kochia prostrata* (L.) Schrad. and *Cleistogenes songorica* (Roshev.) Ohwi.

Long-term simulated N deposition experiments were established in the desert steppe in December 2015. Four N-addition treatments were applied, i.e., control treatment (N0, no N addition), low N addition treatment (N30, 30 kg N/(hm^2·a)), mediate N addition treatment (N50, 50 kg N/(hm^2·a)), and high N addition treatment (N100, 100 kg N/(hm^2·a)). To mirror the natural seasonal N deposition pattern from May to September, we mixed NH_4NO_3 with purified water (10.0 L per plot; under N0 treatment, plots received only purified water) and sprinkled evenly on each plot using a sprayer to simulate wet deposition. From October to April of the next year, NH_4NO_3 was mixed with soil (1.0 kg sand per plot; under N0 treatment, plots received only soil) and broadcasted evenly by hand to simulate dry deposition. N was applied once a month at the beginning of the month. The monthly N application rate was determined by the percentage of the average monthly precipitation in the last 5 years relative to total annual precipitation. We planned the experiments according to a randomized block design with 4 replicate blocks. Each plot had an area of 7.0 m×7.0 m, and the plots were separated by 1-m intervals.

2.2 Plant, soil, and soil microbial sampling

In August 2021, at the peak of the growing season, we randomly arranged three 0.5 m×0.5 m subplots in each plot, the edges of which were parallel to but at least 1 m away from the edges of the plot. We harvested all aboveground plant parts, sorted the plants into species, recorded the species richness and abundance (number of individuals), and dried the plants at 65°C for 48 h to measure the aboveground biomass (AGB) of each species in each subplot. Soil samples were collected using the "five-point method" to a depth of 10 cm. After removing roots and stones and gently mixing the soil, each sample was placed into a sterile plastic bag. One part was used to determine soil physical and chemical properties including, pH, total nitrogen (TN), soil organic carbon (SOC), NH_4^+-N, and NO_3^--N contents (Zha, 1997), and the other was used for the extraction of soil deoxyribonucleic acid (DNA).

2.3 Soil microbial DNA extraction and sequencing

We extracted the microbial community genomic DNA from 0.5 g soil using Soil DNA Purification Mini Kit (Omega Bio-Tek, Norcross, USA) according to the manufacturer's instructions. DNA extract was then checked on a 1% agarose gel, and DNA concentration and purity were determined with a NanoDrop 2000 UV spectrophotometer (Thermo Scientific, Wilmington, USA). To determine the soil bacterial and fungal community composition and diversity, we implemented amplicon surveys of the 16S and ITS (Internal Transcribed Spacer) rRNA (ribosomal ribonucleic acid). The V3-V4 hypervariable regions of the 16S rRNA gene were amplified using the 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') primer sets. The ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) primers were used to amplify the ITS1 region of the fungal rRNA. Polymerase chain reaction (PCR) amplification of the 16S and ITS rRNA genes was performed through the following steps: an initial denaturation at 95°C for 3 min followed by 27 denaturing

cycles at 95°C for 30 s each, annealing at 55°C for 30 s and extension at 72°C for 45 s, a single extension at 72°C for 10 min, and a terminal temperature of 4°C. PCR mixtures contained of 5×TransStart FastPfu buffer (4 μL), 2.5 mM dNTPs (deoxyribonucleoside triphosphates) (2 μL), 5 μM forward primer (0.8 μL), 5 μM reverse primer (0.8 μL), TransStart FastPfu DNA polymerase (0.4 μL), 10 ng template DNA, and finally up to 20 μL ddH_2O. PCR tests were performed in triplicate. We extracted PCR product from a 2% agarose gel, purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, USA) according to the manufacturer's instructions, and quantified using a Quantus™ Fluorometer (Promega, Madison, USA).

We pooled the purified amplicons in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300/NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols established by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw reads were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (PRJNA799672).

The raw 16S and ITS rRNA gene sequencing reads were de-multiplexed and quality-filtered using FASTP v.0.20.0 (Chen et al., 2018) and merged using FLASH v.1.2.7 (Magoc and Salzberg, 2011) with the following criteria: the 300-bp reads were truncated at any site with an average quality score <20 over a 50-bp sliding window, truncated reads shorter than 50 bp were discarded, and reads containing ambiguous characters were also discarded; we only assembled overlapping sequences longer than 10 bp according to their overlapped sequences, set the maximum mismatch ratio of the overlapping region to 0.2, discarded reads that could not be assembled; distinguished the samples according to the barcode and primers, and adjusted the sequence direction through exact barcode matching and 2-nucleotide mismatches in the primer matching process.

Operational taxonomic units (OTUs) with 97% similarity cut-off values (Stackebrandt and Goebel, 1994; Edgar, 2013) were clustered using UPARSE v.7.1 (Edgar, 2013), and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed using the RDP Classifier v.2.2 (Wang et al., 2007) against the 16S and ITS rRNA databases with a confidence threshold of 0.7.

2.4 Statistical analysis

Microsoft Excel 2019 and R software were used for the statistical data analyses. All figures were illustrated using Origin 2021 software. The soil microorganism alfa-diversity and redundancy analysis (RDA) data were analyzed on the online tool of Majorbio Cloud Platform (https://cloud.majorbio.com/page/tools/). The significant differences among N-addition levels were obtained separately for the plant biomass, diversity index, and soil variables using Tukey's honestly significant difference test (P<0.05). We used nonmetric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA) to determine the influences of N deposition on the composition of plant and soil microbial communities between treatments at the genus level based on Bray–Curtis distances.

3 Results

3.1 Soil physical-chemical properties, plant diversity, and community composition

N-addition treatments did not affect the AGB, while the plant community composition was significantly altered (P<0.01; Table 1; Fig. 1a). Compared with N0 treatment, N100 treatment significantly decreased the biomass of perennial grass *S. breviflora*, but significantly increased the biomass of annual and biennial *N. pectinate* (P<0.05; Table 1). However, N deposition did not significantly affect other plant biomass (Table 1). The biomass of perennial grasses gradually decreased with increasing N additions and was replaced by annuals and biennials (Fig. 1a). N deposition did not significantly affect biomass of perennial forbs, shrubs, and semi-shrubs (Fig. 1b). Compared with N0 treatment, N deposition did not significantly affect plant Shannon index, which tended to increase under N30 and N50 treatments (Fig. 1c). Compared with N0 treatment, the Shannon index of the plants under N100 treatment showed a decreasing trend, but did not

reach a significant level (Fig. 1c). Compared with N30 and N50 treatments, Shannon index of plants under N100 treatment significantly decreased (P<0.05; Fig. 1c).

Table 1 Effects of nitrogen (N) treatments on plant biomass

Plant species	N0	N30	N50	N100
	(g/m^2)			
Stipa breviflora Griseb.	90.14±2.35^{a}	73.66±12.67^{ab}	53.07±4.61^{ab}	45.94±7.15^{b}
Neopallasia pectinata (Pall.) Poljak	37.22±11.65^{b}	26.64±6.41^{b}	80.77±10.03^{ab}	106.81±14.21^{a}
Artemisia scoparia Waldst. et Kit.	6.13±3.25^{a}	28.34±13.06^{a}	6.90±2.12^{a}	3.14±1.47^{a}
Cleistogenes songorica (Roshev.) Ohwi.	10.49±3.02^{a}	10.30±1.32^{a}	15.09±1.40^{a}	6.66±2.91^{a}
Kochia prostrata (L.) Schrad.	5.14±0.75^{a}	8.87±5.16^{a}	0.95±0.73^{a}	10.24±7.08^{a}
Convolvulus ammannii Desr.	1.67±0.94^{a}	4.19±1.20^{a}	1.61±1.10^{a}	0.59±0.43^{a}
Agropyron mongolicum Keng	2.55±0.97^{a}	1.95±0.96^{a}	0.10±0.06^{a}	0.91±0.36^{a}
Allium tenuissimum L.	0.61±0.34^{a}	3.46±1.08^{a}	1.05±0.58^{a}	0.72±0.49^{a}
Stipa krylovii Roshev.	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.28±0.21^{a}
AGB	153.95±5.81^{a}	157.41±8.25^{a}	159.53±13.56^{a}	175.27±16.95^{a}

Note: Different lowercase letters within the same row indicate significant differences among treatments at P<0.05 level. Mean±SE. N0, control; N30, 30 kg N/(hm^2•a); N50, 50 kg N/(hm^2•a); N100, 100 kg N/(hm^2•a); AGB, aboveground biomass. The abbreviations are the same in the following tables.

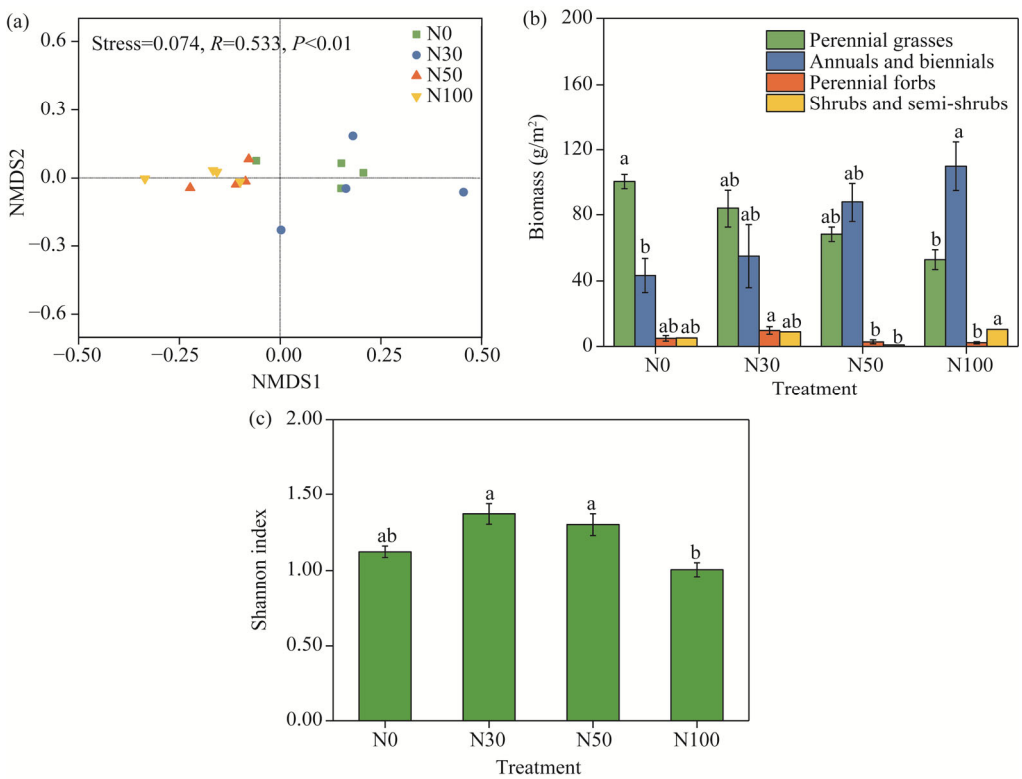

Fig. 1 Biomass of plant functional group compositions (a), non-metric multidimensional scaling (NMDS) plot illustrating distances between plant community compositions (b), and plant Shannon index (c) under different N treatments. In Figure 1a, R value is the ANOSIM (analysis of similarities) statistic R, and P value is the significance from permutation; in Figure 1b, different lowercase letters within the same treatment indicate significant differences among different plant functional groups at P<0.05 level; in Figure 1c, different lowercase letters indicate significant differences among different N treatments at P<0.05 level. N0, control; N30, 30 kg N/(hm^2•a); N50, 50 kg N/(hm^2•a); N100, 100 kg N/(hm^2•a). Bars are standard errors. The abbreviations are the same in the following figures.

Soil pH tended to decrease gradually with increasing N addition, but did not reach significant levels (Table 2). Conversely, soil SOC showed an increasing trend with the amount of N added (Table 2). N addition did not significantly affect soil TN and C/N ratio, but increased soil NH_4^+–N and NO_3^--N contents (Table 2). Compared with N0 treatment, N100 treatment significantly increased NH_4^+-N and NO_3^--N contents (P<0.05; Table 2).

Table 2 Effects of N treatments on soil physical-chemical characteristics

Variable	N0	N30	N50	N100
pH	8.38±0.01[a]	8.36±0.06[a]	8.30±0.06[a]	8.20±0.05[a]
TN (g/kg)	1.88±0.14[a]	1.84±0.10[a]	1.83±0.08[a]	1.92±0.12[a]
SOC (g/kg)	18.84±1.09[a]	20.64±1.58[a]	20.91±0.49[a]	21.05±0.69[a]
C/N	10.13±0.51[a]	11.22±0.47[a]	11.47±0.30[a]	11.12±0.43[a]
NH_4^+-N (mg/kg)	1.38±0.14[c]	2.36±0.57[c]	6.15±0.20[b]	14.7±0.90[a]
NO_3^--N (mg/kg)	9.46±1.11[b]	12.72±0.77[b]	26.95±1.26[b]	100.26±10.31a

Note: TN, total nitrogen; SOC, soil organic carbon; C/N, soil organic carbon/total nitrogen. Different lowercase letters within the same row indicate significant differences among different N treatments at P<0.05 level. Mean±SE.

3.2 Soil bacterial and fungal community composition and diversity

The dominant bacteria across all treatments were Actinobacteriota, Proteobacteria, Acidobacteriota, and Chloroflexi, which together accounted for more than 83.46% of the total sequences (Fig. 2a). The relative abundance of Actinobacteriota gradually increased as the amount of N added increased (Fig. 2a). The relative abundance of Proteobacteria showed a gradual decrease from N0 to N50 treatments, while the relative abundance of Proteobacteria under N100 treatment was higher than that under N0 treatment, showing an increasing trend

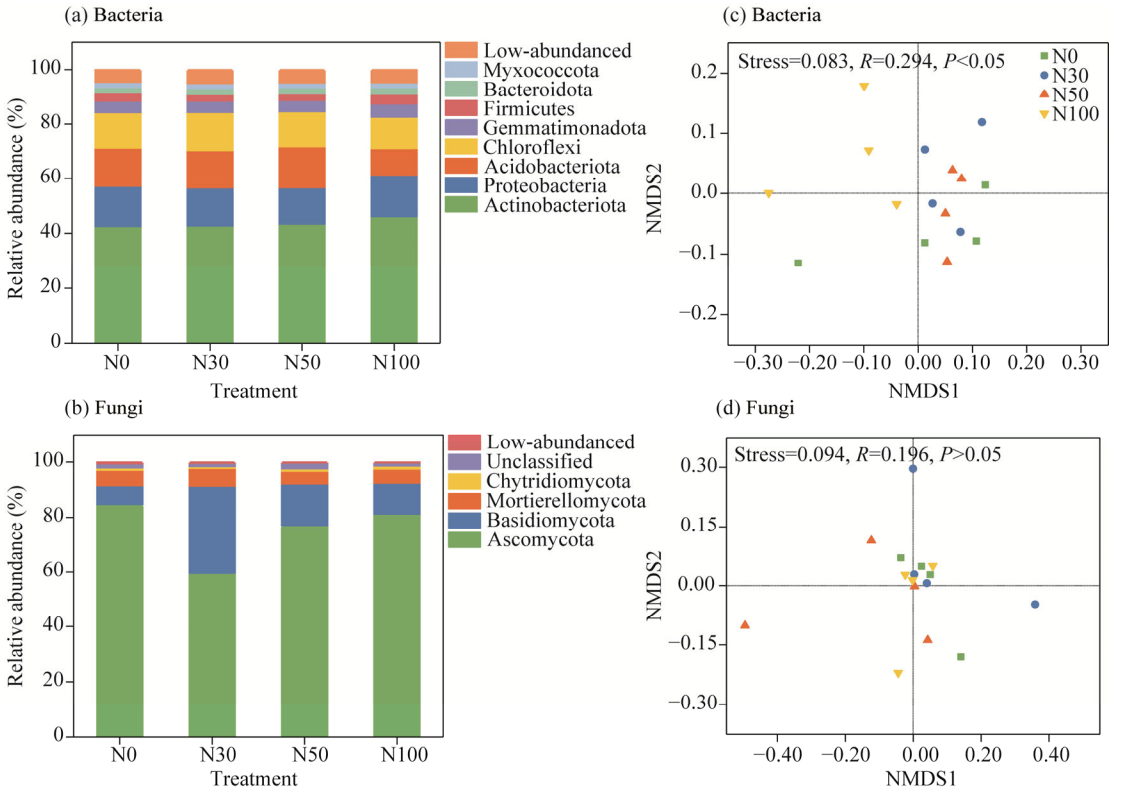

Fig. 2 Relative abundance of dominant bacterial phyla (a), fungal phyla (b), and non-metric multidimensional scaling (NMDS) plot illustrating distances between microbial community composition for bacteria (c) and fungi (d) under different N treatments

(Fig. 2a). NMDS analysis showed significant differences in soil bacterial community composition among different N treatments (P<0.05; Fig. 2b).

The dominant fungi across all treatments were Ascomycota, Basidiomycota, and Mortierellomycota, which together accounted for more than 97.26% of the total sequences (Fig. 2c). Relative abundance of Basidiomycota was the highest under N30 treatment, followed by N50, N100, and N0 treatments (Fig. 2c). Relative abundance of Ascomycota was opposite to Basidiomycota, being the lowest under N30 treatment (Fig. 2c). N deposition did not significantly affect soil fungal community composition in the desert steppe (P>0.05; Fig. 2d).

N deposition did not significantly affect the Shannon index of soil bacterial community, but significantly affected the Chao1 index (Fig. 3a and b). The soil bacterial Chao1 index was consistent with the pattern of change of plant Shannon index. N deposition had no significant effects on Shannon and Chao1 indices of soil fungal communities (Fig. 3c and d).

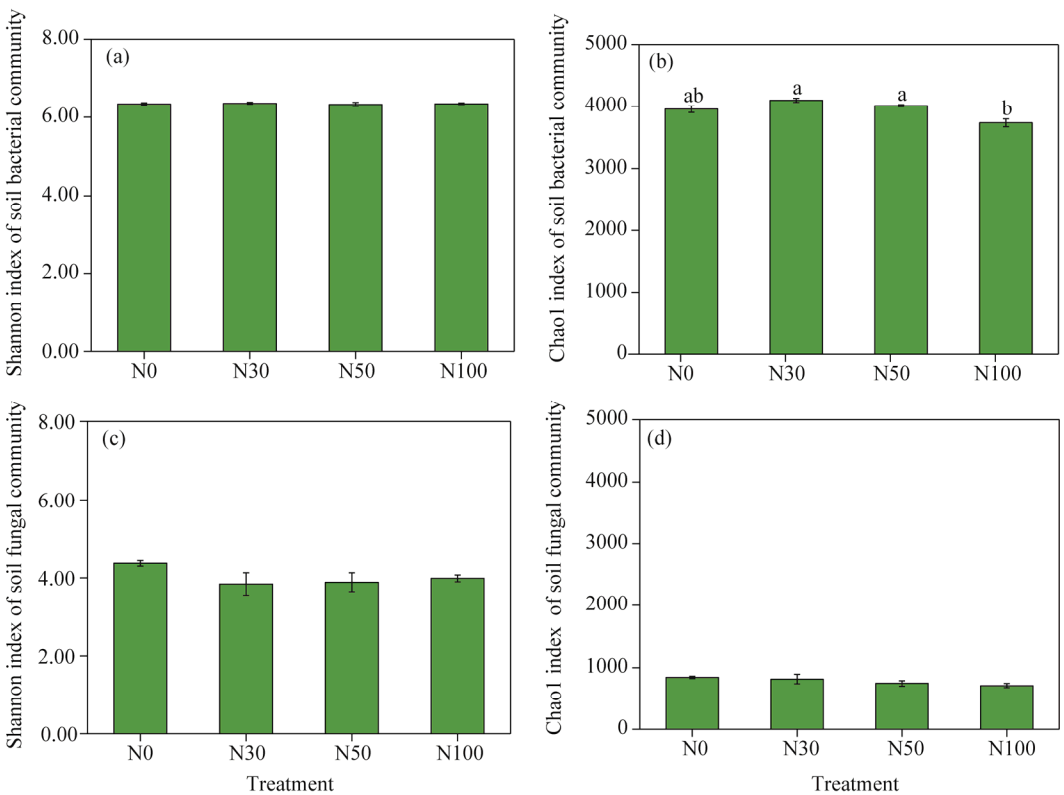

Fig. 3 Shannon index and Chao1 richness for soil bacterial community (a and b) and fungal community (c and d) under different N treatments. Different lowercase letters indicate significant differences among different N treatments at P<0.05 level. Bars are standard errors.

3.3 Soil and plant properties associated with microbial community structure

The first axis explained 22.25% and 23.50% changes in structure of bacterial and fungal community influenced by environmental factor, respectively (Fig. 4a and b). In the soil bacteria RDA biplot, soil pH and plant Shannon index were positively correlated with soil bacterial communities under N0, N30, and N50 treatments (Fig. 4a). On the contrary, soil pH and plant Shannon index were negatively correlated with soil bacterial community under N100 treatment (Fig. 4a). Soil pH was negatively correlated with Proteobacteria, Gemmatimonadota, and Bacteroidota, but positively correlated with Chloroflexi (Table S1). NH_4^+-N and NO_3^--N were negatively correlated with soil bacterial communities under N0, N30, and N50 treatments (Fig. 4a). On the contrary, NH_4^+-N and NO_3^--N were positively correlated with soil bacterial

community under N100 treatment (Fig. 4a). NH_4^+-N and NO_3^--N were positively correlated with Actinobacteriota (P<0.05; Table S1). In the soil fungi RDA biplot, NH_4^+-N, NO_3^--N, annuals and biennials, and AGB were positively correlated with soil bacterial communities under all N addition treatments (Fig. 4b). NH_4^+-N, NO_3^--N, annuals and biennials, and AGB were positively correlated with Ascomycota and negatively correlated with Basidiomycota and Mortierellomycota, but none of them reached significance (Table S2). NH_4^+-N and NO_3^--N were negatively correlated with plant Shannon index and perennial grasses (P<0.05; Table S3), but positively correlated with annuals and biennials (P<0.05; Table S3).

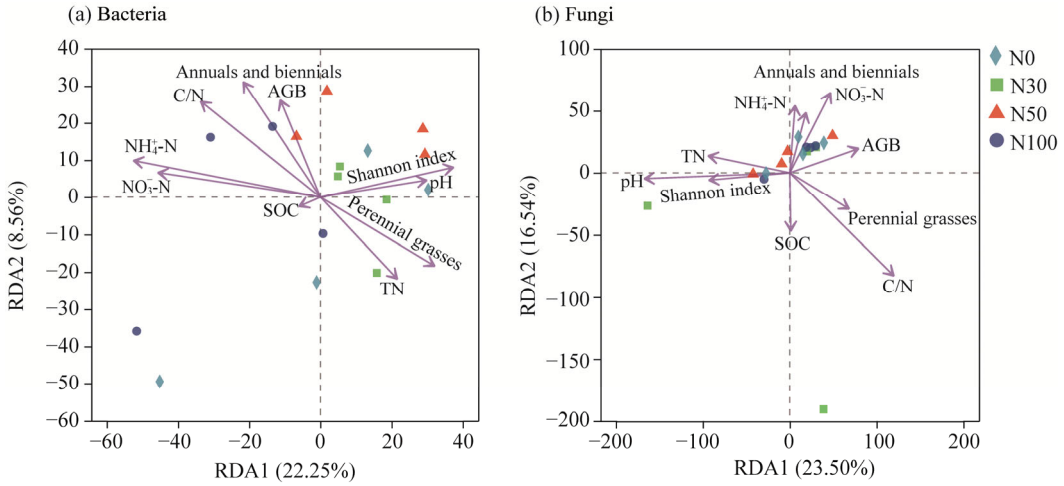

Fig. 4 Redundancy analysis (RDA) of environmental factor with bacteria (a) and fungi (b). AGB, aboveground biomass; TN, total nitrogen; SOC, soil organic carbon; C/N, soil organic carbon/total nitrogen.

4 Discussion

The results of our experiments showed that N deposition in the 6-year experiment did not cause any significant increase in plant AGB, but did change the composition of plant community. Some results of previous studies have shown that the increase in aboveground net primary production as added N stabilizes after approximately 10.5–12.0 g N/(m^2·a) is added in desert steppe regions (Bai et al., 2010; Tang et al., 2017). However, the amount of added N in our study did not exceed this threshold. Therefore, the nonsignificant biomass increases observed herein may have been due to water constraints (Su et al., 2013). This finding is in line with the theoretical predictions of the multiple resource co-limitation theory (Harpole et al., 2011). The effects of N addition on plant communities largely depend on water availability (Delgado-Baquerizo et al., 2013; Ma et al., 2020). Although there were no significant changes in plant AGB, there were significant changes in plant community composition. N addition enhanced the AGB of the annual *N. pectinata*. In contrast to the strong positive effects of N addition on the *N. pectinata* biomass, N detrimentally affected the AGB of the perennial grass *S. breviflora*. Previous studies have also reported that fast-growing annuals, which are usually abundant only in the early stages of grassland succession, can almost completely replace perennials in mature sites (Bai et al., 2010). The rapid growth of annuals is facilitated by their species-specific traits, including their abundant seed production and rapid growth. In contrast, the decline in the abundance of perennials could be attributable to their conservative resource-use strategies (Bai et al., 2010). Moreover, another ecological adaptive strategy, the r/K selection theory (Gadgil and Solbrig, 1972), might be reflected in the results; forbs that grow faster as r-strategists increased, while native perennial grasses that grow slowly as K-strategists decreased (Ma et al., 2020). This may be the main reason for the absence of significant changes in plant alfa-diversity in this study. However, NO_3^--N and NH_4^+-N were

significantly negatively correlated with plant Shannon index, and prolonged or higher concentrations of N deposition may lead to a significant decline in desert steppe plant diversity.

N addition significantly increased soil available N, which is important limiting factor for microorganisms in the desert steppe (Wang et al., 2020). The low soil N content in desert steppe ecosystems may not only restrict the proliferation and metabolism of soil microorganisms, but also limit the growth of vegetation (Sha et al., 2021). The experimental results obtained under all treatments showed that N addition did not significantly decrease the soil microbial alfa-diversity. In addition to the above-mentioned reasons for water restriction, it may also be that the added rates of N are much higher than that of environment of native inhabitants evolved, because those inhabitants had adapted to the low N environment (Harrington et al., 2001; Peng et al., 2020). This conclusion can also be proved by the fact that the plant biomass did not increase significantly; the change of soil pH caused by N is the most important factor affecting the response of microbial diversity (Wang et al., 2023). Most research has suggested that N deposition decreases soil pH, which weakens the relationship between plants and soil microbes (Li et al., 2018). However, in the present study, soil pH was not significantly affected, and the main effect came from the accumulation of inorganic N. NO_3^--N and NH_4^+-N were significantly and positively correlated with Actinobacteriota and annuals and biennials biomass. N addition increased the relative abundances of Actinobacteriota and annuals and biennials, which could be partially explained by copiotrophic bacteria life history strategies (Dai et al., 2018). Soil microbial diversity findings regarding its responses to N inputs were consistent with those reported in other desert steppe regions (McHugh et al., 2017; Huang et al., 2021), However, in this work, the soil microbial community composition (beta-diversity) was affected by different N-addition amounts. Plant-mediated effects on soil microbial communities may be driven by the quantity and quality of available resources (plant litter and root exudates) or the synchrony between vegetation and microorganisms (Chen et al., 2021). Plants and soil microbes can have direct co-evolutionary relationships, and their relationships constitute important systems (Keymer and Lankau, 2017). The pattern of change of Shannon index of plant community and Chao1 index of soil bacteria in this study was consistent. The composition of plant community (beta-diversity) changes significantly, and the composition of bacteria also changes at the same time, which may be mainly due to the co-evolution between plants and microorganisms. The desert steppe soil bacterial community changed co-evolution with the plant community, but neither fungal community structure nor diversity was significantly affected by N deposition. Studies from arid and semi-arid grasslands have shown that drought promotes the destabilizing properties of soil bacteria but not fungi and thus has a prolonged effect on bacterial communities and their co-occurrence networks via changes in the vegetation composition and the resultant reductions in soil moisture (de Vries et al., 2018; Fan et al., 2020; Wang et al., 2020). These results contribute to a comprehensive understanding of the effects of atmospheric N deposition on biodiversity and the mechanism underlying plant-soil-microbiome interaction in the desert steppes.

5 Conclusions

The data from this study indicated that in nutrient-poor and arid desert steppe ecosystems, plant AGB did not significantly increase after a 6-year of N deposition. Simultaneously, N deposition did not decrease alfa-diversity of plants and microorganisms in the desert steppes, and low N addition had a positive effect on them. However, N deposition significantly affected beta-diversity of plant and bacterial communities, but did not significantly impact fungal communities. Plants and soil bacterial communities co-evolved, but they did not significantly impact fungal communities. These findings help to understand the mechanisms of atmospheric N deposition effects on the ecological health and function of the desert steppes.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All authors contributed to the conceptualization; Methodology, formal analysis, and writing - review and editing: HE Ye, MEI Hong, XU Xuehui, LIANG Zhiwei, JIANG Na, TU Nare, WU Zhendan; Writing - original draft preparation: HE Ye. All authors approved the manuscript.

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Appendix

Table S1 Correlation between environmental factors and dominant bacterial phyla

Variable	Actino-bacteriota	Proteo-bacteria	Acido-bacteriota	Chloro-flexi	Gemmati-monadota	Firmi-cutes	Bacteroi-dota	Myxoco-ccota
pH	–0.34	–0.52*	0.45	0.70**	–0.55*	–0.44	–0.71**	–0.09
TN	0.35	–0.11	–0.26	0.25	–0.16	0.07	–0.22	0.26
SOC	0.32	0.06	–0.33	0.02	0.13	0.30	0.02	0.18
C/N	–0.06	0.20	–0.06	–0.30	0.33	0.26	0.28	–0.15
NH_4^+-N	0.57*	0.09	–0.38	–0.48	0.35	0.26	0.24	–0.10
NO_3^--N	0.55*	0.12	–0.38	–0.42	0.25	0.28	0.22	–0.13
Shannon index	–0.24	–0.16	0.17	0.41	–0.14	–0.38	–0.06	0.20
AGB	0.06	–0.06	0.02	–0.27	0.16	–0.01	0.08	–0.22
Perennial grasses	–0.38	0.12	0.16	0.20	–0.14	0.08	–0.21	0.11
Annuals and biennials	0.22	–0.01	–0.07	–0.38	0.19	0.00	0.26	–0.18

Note: *, $P<0.05$ level; **, $P<0.01$ level. AGB, aboveground biomass; TN, total nitrogen; SOC, soil organic carbon; C/N, soil organic carbon/total nitrogen. The abbreviations are the same in the following tables.

Table S2 Correlation between environmental factors and dominant fungal phyla

Variable	Ascomycota	Basidiomycota	Mortierellomycota	Chytridiomycota
pH	–0.19	0.20	–0.24	0.02
TN	0.08	–0.14	0.36	0.48
SOC	–0.12	0.05	0.31	0.47
C/N	–0.22	0.24	–0.15	–0.04
NH_4^+-N	0.15	–0.10	–0.21	0.31
NO_3^--N	0.25	–0.22	–0.16	0.43
Shannon index	–0.40	0.35	0.02	–0.25
AGB	0.14	–0.12	–0.05	–0.23
Perennial grasses	0.06	–0.12	0.39	–0.18
Annuals and biennials	0.20	–0.16	–0.19	–0.02

Table S3 Correlation between soil properties and plant community

Plant community	pH	TN	SOC	C/N	NH_4^+-N	NO_3^--N
Shannon index	0.41	–0.29	–0.14	0.16	–0.53*	–0.58*
AGB	–0.30	–0.25	–0.34	–0.08	0.36	0.28
Perennial grasses	0.24	0.12	0.16	0.02	–0.73**	–0.62**
Annuals and biennials	–0.46	–0.19	–0.29	–0.09	0.62**	0.55*

Note: *, $P<0.05$ level; **, $P<0.01$ level.